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Title: Contribution to the detection of staphylococcal enterotoxin. II. Concerning the effect of staphylococcal enterotoxins on nematodes (Beitrag zum Nachweis von Staphylokokkenenterotoxin. II. Uber die Wirkung des Staphylokokkenenteroxins auf Nematoden.)

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July 1969

The difficulty which is encountered in the case of the detection of staphylococcal enterotoxin, especially since Dack (1931) and Jordan (1933) demonstrated that the conventional laboratory animals are not suitable for this purpose, had led to the introduction of numerous methods of detection. Most of these methods today are of historical significance only.

Of the methods of detection that have been introduced since the earliest studies, the serological methods merit particular attention (Casman, 1958, and Bergdoll et al., 1959). The employment of serological techniques, however, is complicated by the heterogeneous nature of staphylococcal enterotoxins (Casman et al., 1963; Bergdoll et al., 1965) and the technical difficulties which are inherent to the tests themselves. As a result, the development of a specific, sensitive biological test for the detection of staphylococcal enterotoxin would certainly be a worthwhile endeavor.

The influence of staphylococcal enterotoxin on various protozoa and lower forms of metazoa was studied by Intosh and Duggan (1965) who were not able to demonstrate a specific sensitivity in this regards. The relative failure of existing method led, as a result, to the consideration of nematodes as test animals for the assay of staphylococcal enterotoxin.

The communication of del Valle (1960) concerning successful results using Rhabditis strongyloides, however, were refuted in the same year by Sugiyama (1960). Using the same study format but employing highly purified enterotoxin, the latter was unable to demonstrate a specific lethal effect on nematodes.

Because of the enormous advantage that would be offered by the use of macroscopically visible nematodes, we set ourselves to the task of ascertaining the sensitivity of nematodes to purified staphylococcal enterotoxin. In order to accomplish this, we employed nematodes which had been previously used by other investigators (Frick, 1963; Kampfe, 1963a, b, and 1964).

MATERIALS

Test Animals

For these studies, the nematodes Rhabditis oxycerca de Man, 1895, and Panagrellus redivivus Goody, 1945, were employed*.

Culturing of the nematodes was carried out using the method of Kampfe (1963a, 1964) on oatmeal mash in specimen tubes with covers or in reagent bottles at temperatures of 21-25°C in the dark. The normal development of the nematodes was characterized by the fact that the nematodes left the semi-solid substrate and creeped up along the glass walls. For each study, freshly washed, young, normally developed cultures were employed.

Staphylococcal Enterotoxin

For the studies, an enterotoxin type A or B of Strain S-6 which was 20 % pure using the method of Bergdoll et al. (1959, 1960) was used for the most part.

METHODS

During the preliminary studies, the sensitivity of the nematodes to staphylococcal enterotoxin was ascertained. The reduction in resistance after washing with distilled water or

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* We wish to thank Dr. Kampfe of the Zoological Institute of Ernst-Moritz University, Greifswald for supplying the two strains.

with physiological saline was carried out experimentally during 24 - 72 hours of fasting. In addition, the following factors were studied: storage at different pH values, various molecular concentrations of $Na_2HPO_4 \cdot 12\ H_2O$, different percentages of HCl, and the effect of enzymatically digested proteins (casamino acids, Difco, and NZ-amine). Also studied were the effects of bacterial proteinase and "Dessau" hylase, a highly purified hyaluronidase preparation from bull testes.

Generally, the tests were performed using 20 - 25 worms and 1 ml of the solution to be tested in a weighing dish 2.5 x 3.0 cm.

The lyophilized staphylococcal enterotoxin was employed in concentrations ranging from 10,000 to 22,000 µg per ml in the various studies.

Under a stereo-microscope (15 X magnification) or by eye only, the movement of the nematodes was observed at fixed intervals up to 48 hours.

The onset of the initial damage was established using the single-step tables of Kampfe (1963 b):

(1) Light damage: movements affected with distinct tendency of the animal to curl.

(2) Distinct damage: Weak, often only occasional movement often with convulsive movement of the body.

(3) Strong irreversible damage: no movement; the animals are spread out.

All of the controls were carried out for the same period of time and under the same conditions of pH, acidity, etc. except in the absence of enterotoxin.

obtained in the studies on the effect of bacterial protease, 1 - 5 mg per ml, and purified staphylococcal enterotoxin, 5×10^3 - 22×10^3 µg per ml, on fasting nematodes.

As already mentioned, the preliminary treatments produced no significant increase in the sensitivity of the nematodes to staphylococcal enterotoxin. For that reason, the direct effect of several concentrations of purified staphylococcal enterotoxin type A as well as B), dissolved in physiological saline, was determined. 20-25 worms were taken from an active population without previous treatment and placed in separate weighing bottles. They were exposed to 10, 100 and 1000 µg per ml of dissolved staphylococcal enterotoxin (type A as well as type B) and observed for 96 hours.

From Fig 1, it can be seen that the control population exhibited the highest percentage of motile worms (97.7 %) and the smallest percentage of dead worms (1.1 %). The sample treated with 1,000 µg per ml of staphylococcal enterotoxin exhibited 8.9 % mortality and in comparison with both of the preceding toxin concentrations, had a higher percentage of motile worms.

In order to be able to even more clearly demonstrate the ultimate toxic or trophic effect of purified staphylococcal enterotoxin on nematodes, in another series of studies, stronger enterotoxin concentrations (1000 - 5000 µg per ml) were employed.

In Fig. 2 are presented curves which show the effect of increasing doses of staphylococcal enterotoxin (1000 to 5000 µg per ml of distilled water) on the alterations in the movement of the nematodes within 48 hours. The courses of the curves show

that up to the third hour and after 24-48 hours, relatively the same values, namely between 80 and 100 movements per minute, are obtained. The middle of the test period is characterized by a light reduction in the mobility of the test worms (10-25 %).

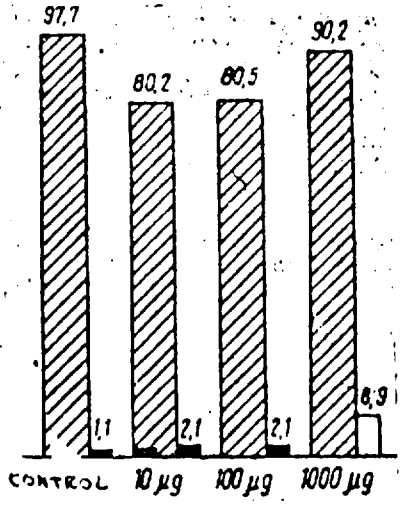

Fig. 1. The effect of staphylococcal enterotoxin (type B), 20 % purified, on Rhabditis oxycera within 96 hours.

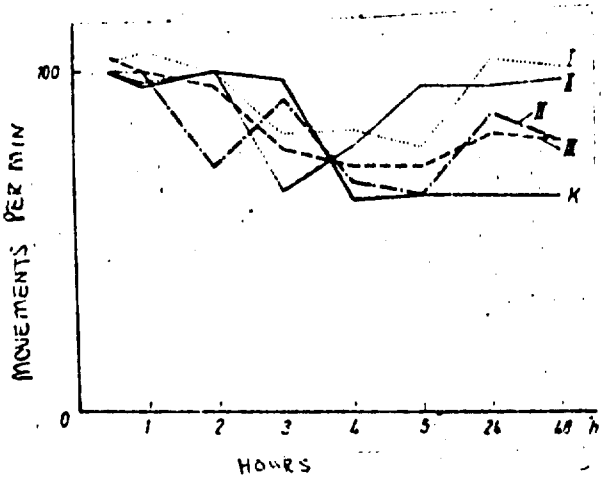

Fig 2. The effect of 20 % purified staphylococcal enterotoxin, type A, on Rhabditis oxycera at pH 6.5 within 48 hours. I: 1000 µg per ml distilled water; II: 2000 µg per ml; III 3,000 µg per ml; IV 5,000 µg per ml; control: water only.

Another important indication of the toxic or trophic effect of purified staphylococcal enterotoxin on the nematodes is the development of young forms (Larvae). In our studies, observations on the number of larvae and their mortality demonstrated that increasing the concentration of enterotoxin did not lead to significant graduated differences in the number of motile and dead larvae. In the case of an enterotoxin concentration of 10 µg per ml, the decrease in dead larvae amounted to 15 %; at 100 µg per ml, it was 10 %; and at 1000 µg per ml, it was still 10 %. This fact speaks out against the thesis concerning the toxic effects of staphylococcal enterotoxin on nematodes of the order "Strongyloides" (del Valle, 1960, and Angelotti, 1963).

The absence of a clearly discernable increase in the number of larvae with increases in the toxin concentration during the studies carried out by us provided no reason for the increases which had been noted by Chang and Hall with regard to a trophic effect on development of nematodes by staphylococcal enterotoxin.

In several series of experiments, the correlation between motility of the nematodies and the pH value (4.5 - 10.5) was studied. One was able to detect statistically a decrease in the degree of motility with increases in pH.

The observations of the oxygen requirements of both nematodes species in ampoules sealed under vacuum and simply closed weighing bottles showed a correlation between oxygen deficiency and the rapid increase in the dynamics of motility.

Examination for an eventual toxic effect by 1-5 % solutions of nutrient medium such as casamino acids (Difco) and NZ-amine, did not confirm the observations made by Sugiuama (1960).

SUMMARY

With the goal of developing a biological assay method for staphylococcal enterotoxin, the effect on the development of nematodes of the order "Strongyloides", Rhabditis oxycerca de Man and Panagrellus redivivus Goody was examined.

After preparation by fasting and treatment with 0.02 - 0.2 M $Na_2HPO_4 \cdot 12\ H_2O$, 750 - 2,250 units per ml of Hylase, or 1 - 5 mg per ml bacterial protease, or with untreated mixed cultures (adults and larvae), significant resistance was demonstrated in the case of both nematodes species when they were tested against 20 % purified staphylococcal enterotoxin (type A as well as B) in concentrations from 1,000 to 22, 000 µg per ml. It is concluded that these animals are unsuitable for the detection of staphylococcal enterotoxin.

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